

WHAT IS CLAIMED IS:

- 1 1. A method for identifying an agent capable of modulating the interaction
2 between a transcription factor and a Stat protein comprising:
3 (a) providing said transcription factor or a fragment thereof;
4 (b) providing a Stat protein fragment comprising a region within from
5 about residue 107 to about residue 377 of said Stat protein;
6 (c) incubating mixtures of said transcription factor or fragment thereof and
7 said Stat protein fragment with and without said agent;
8 (d) detecting the extent of interaction between said transcription factor or
9 fragment thereof and said Stat protein fragment in each of said
10 mixtures; and
11 (e) identifying an agent as capable of modulating said interaction as one
12 which alters said extent of interaction.
- 1 2. The method of claim 1 wherein said Stat protein fragment comprises the
2 coiled-coil domain of said Stat protein and the first three β -strands of the
3 DNA-binding domain of said Stat protein.
- 1 3. The method of Claim 1 wherein said Stat protein is selected from the group
2 consisting of Stat1, Stat2, Stat3, Stat4, Stat5 and Stat6.
- 1 4. The method of Claim 3 wherein said Stat protein is Stat3.
- 1 5. The method of Claim 4 wherein said Stat3 protein fragment is selected from
2 the group consisting of (1) the region comprising about residue 107 to about
3 residue 358, (2) the region comprising about residue 130 to about residue 358,
4 (3) the region comprising about residue 155 to about residue 377, (4) the
5 region comprising about residue 193 to about residue 377, (5) the region
6 comprising about residue 249 to about residue 377, and (6) the region

7 comprising about residue 282 to about residue 377.

- 1 6. The method of claim 4 wherein said Stat3 protein fragment is selected from
2 the group consisting of SEQ ID NO:9, SEQ ID NO:14, SEQ ID NO:15, SEQ
3 ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20,
4 SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24 and SEQ ID
5 NO:25.
- 1 7. The method of Claim 1 wherein said Stat protein or fragment thereof is labeled
2 with a detectable label.
- 1 8. The method of Claim 7 wherein said label is a GST fusion sequence or an
2 epitope tag.
- 1 9. The method of Claim 1 wherein said transcription factor is selected from the
2 group consisting of the JUN, the FOS, and the ATF families of transcription
3 factors.
- 1 10. The method of claim 9 wherein said JUN transcription factor is selected from
2 the group consisting of c-Jun, JunB and JunD.
- 1 11. The method of claim 9 wherein said FOS transcription factor is selected from
2 the group consisting of c-Fos, FosB, Fra-1 and Fra-2.
- 1 12. The method of claim 9 wherein said ATF transcription factor is selected from
2 the group consisting of ATF-1, ATF-2, ATF-3 and ATF-4.
- 1 13. The method of claim 9 wherein said fragment comprises the COOH-terminal
2 region of said transcription factor.

1 14. The method of claim 10 wherein said fragment comprises the bZIP region of
2 said transcription factor.

1 15. The method of Claim 9 wherein said transcription factor is c-Jun.

1 16. The method of claim 15 wherein said fragment comprises the region of about
2 residue 105 to about residue 334 of c-Jun.

1 17. The method of claim 15 wherein said fragment comprises the region of about
2 residue 105 to about residue 263 of c-Jun.

1 18. The method of Claim 1 wherein said transcription factor or fragment thereof is
2 labeled with a detectable label.

1 19. The method of Claim 18 wherein said label is a radiolabel.

1 20. The method of Claim 1 wherein said detection of the extent of interaction is
2 performed by GST protein association assay, coimmunoprecipitation, or the
3 yeast 2-hybrid system..

1 21. The method of Claim 4 wherein said agent modulates the interaction between
2 said transcription factor and said Stat3 protein at residues of said Stat3 protein
3 selected from the group consisting of residues 130-154, residues 343-358, and
4 the combination thereof.

1 22. The method of Claim 1 wherein said agent is a Stat protein antagonist.

1 23. The method of Claim 15 wherein said agent modulates the interaction between

2 said c-Jun and said Stat protein comprising about residue 105 up to about 334
3 of c-Jun.

1 24. The method of Claim 23 wherein said agent modulates the interaction between
2 said c-Jun and said Stat protein comprising about residue 105 to about 263 of
3 c-Jun.

1 25. The method of Claim 24 wherein said c-Jun residues are 105-263.

1 26. A method for identifying an agent capable of modulating the transcriptional
2 cooperation between a transcription factor and a Stat protein comprising

- 3 (a) providing a transiently transfected cell bearing a Stat-inducible reporter
4 gene;
- 5 (b) introducing into said cell a transcriptionally cooperative combination
6 of a wild-type Stat protein or mutant thereof, and a wild-type
7 transcription factor or mutant thereof;
- 8 (c) inducing the expression of said reporter gene;
- 9 (d) determining the extent of expression of said reporter gene in the
10 presence and absence of said agent; and
- 11 (e) identifying an agent capable of modulating said interaction as one able
12 to alter the expression of said reporter gene.

1 27. The method of claim 26 wherein said Stat protein or mutant thereof comprises
2 the coiled-coil domain of said Stat protein and the first three β -strands of the
3 DNA-binding domain of said Stat protein.

1 28. The method of Claim 26 wherein said Stat protein is selected from the group
2 consisting of Stat1, Stat2, Stat3, Stat4, Stat5 and Stat6.

1 29. The method of Claim 28 wherein said Stat protein is Stat3.

1 30. The method of Claim 29 wherein said agent modulates the interaction between
2 said transcription factor and said Stat3 protein at residues of said Stat3 protein
3 selected from the group consisting of residues 130-154, residues 343-358, and
4 the combination thereof.

1 31. The method of claim 29 wherein said Stat3 mutant has at least one mutation in
2 a region of the native Stat3 sequence at positions selected from the group
3 consisting of residues 130-154, residues 343-358, and the combination thereof.

1 32. The method of claim 31 wherein said Stat3 mutant is selected from the group
2 consisting of Stat3(L148A) (SEQ ID NO:30), Stat3(V151A) (SEQ ID
3 NO:31), and Stat3(T346A, K348A, R350A) (SEQ ID NO:29).

1 33. The method of Claim 26 wherein said Stat protein or mutant thereof is labeled
2 with a detectable label.

1 34. The method of Claim 33 wherein said label is a GST fusion sequence or an
2 epitope tag.

1 35. The method of Claim 26 wherein said transcription factor is selected from the
2 group consisting of the JUN, the FOS, and the ATF families of transcription
3 factors.

1 36. The method of claim 35 wherein said JUN transcription factor is selected from
2 the group consisting of c-Jun, J^{un}B and JunD.

1 37. The method of claim 35 wherein said FOS transcription factor is selected from

2 the group consisting of c-Fos, FosB, Fra-1 and Fra-2.

1 38. The method of claim 35 wherein said ATF transcription factor is selected from
2 the group consisting of ATF-1, ATF-2, ATF-3 and ATF-4.

1 39. The method of Claim 36 wherein said transcription factor is c-Jun.

1 40. The method of Claim 26 wherein said transcription factor or fragment thereof
2 is labeled with a detectable label.

1 41. The method of Claim 40 wherein said label is a radiolabel.

1 42. The method of Claim 26 wherein said agent modulates the transcriptional
2 cooperation between said transcription factor and said Stat3 protein at residues
3 of said c-Jun protein selected from the group consisting of residues about 105
4 up to about 334.

1 43. The method of Claim 26 wherein said agent is a Stat protein antagonist.

1 44. The method of Claim 33 wherein said c-Jun interaction regions is within
2 residues about 105 up to about 334.

1 45. The method of Claim 44 wherein said c-Jun interaction regions is within
2 residues about 105 and up to about 263.

1 46. The method of Claim 43 wherein said residues are 105-263.

1 47. The method of claim 39 wherein said c-Jun mutant has at least one mutation in
2 a region of the native Stat3 sequence within positions 105 and 263, or 105 and

1 48. A method for identifying a mutant in a molecule selected from the group
2 consisting of a transcription factor, a Stat protein, and the combination thereof,
3 said mutant capable of modulating the transcriptional cooperation between
4 said transcription factor and a Stat protein comprising
5 (a) providing a transiently transfected cell bearing a Stat-inducible
6 reporter gene;
7 (b) introducing into said cell a wild-type Stat protein, fragment or
8 mutant thereof; and a wild-type transcription factor, fragment
9 or mutant thereof, wherein at least one of said introduced Stat
10 protein or transcription factor is mutant or a fragment;
11 (c) inducing the expression of said reporter gene;
12 (e) determining the extent of expression of said reporter gene
13 compared to said extent in a cell having a wild-type form of at
14 least one of said mutant transcription factor or said mutant Stat
15 protein; and
16 (f) identifying a mutant as one capable of modulating said
17 interaction as one able to alter the expression of said reporter
18 gene.

1 49. The method of claim 48 wherein said Stat protein, fragment or mutant thereof
2 comprises the coiled-coil domain of said Stat protein and the first three β -
3 strands of the DNA-binding domain of said Stat protein.

1 50. The method of Claim 48 wherein said Stat protein is selected from the group
2 consisting of Stat1, Stat2, Stat3, Stat4, Stat5 and Stat6.

1 51. The method of Claim 50 wherein said Stat protein is Stat3.

1 52. The method of Claim 48 wherein said mutation modulates the transcriptional
2 cooperation between said transcription factor and said Stat3 protein at residues
3 of said Stat3 protein selected from the group consisting of residues 130-154,
4 residues 343-358, and the combination thereof.

1 53. The method of claim 51 wherein said Stat3 mutant has at least one mutation in
2 a region of the native Stat3 sequence at positions selected from the group
3 consisting of residues 130-154, residues 343-358, and the combination thereof.

1 54. The method of claim 53 wherein said Stat3 mutant is selected from the group
2 consisting of Stat3(L148A) (SEQ ID NO:30), Stat3(V151A) (SEQ ID
3 NO:31), and Stat3(T346A, K348A, R350A) (SEQ ID NO:29).

1 55. The method of Claim 51 wherein said Stat protein or mutant thereof is labeled
2 with a detectable label.

1 56. The method of Claim 55 wherein said label is a GST fusion sequence or an
2 epitope tag.

1 57. The method of Claim 48 wherein said transcription factor is selected from the
2 group consisting of the JUN, the FOS, and the ATF families of transcription
3 factors.

1 58. The method of claim 57 wherein said JUN transcription factor is selected from
2 the group consisting of c-Jun, JunB and JunD.

1 59. The method of claim 57 wherein said FOS transcription factor is selected from
2 the group consisting of c-Fos, FosB, Fra-1 and Fra-2.

1 60. The method of claim 57 wherein said ATF transcription factor is selected from
2 the group consisting of ATF-1, ATF-2, ATF-3 and ATF-4.

1 61. The method of Claim 58 wherein said transcription factor is c-Jun.

1 62. The method of Claim 48 wherein said transcription factor or mutant thereof is
2 labeled with a detectable label.

1 63. The method of Claim 62 wherein said label is a radiolabel.

1 64. The method of Claim 48 wherein said mutation modulates the transcriptional
2 cooperation between said transcription factor and said Stat3 protein at residues
3 of said c-Jun at positions 105-334, or 105-263.

1 65. A Stat protein fragment selected from the group consisting of residues 1-154
2 of Stat3 (SEQ ID NO:8), residues 107-377 of Stat3 (SEQ ID NO:9), residues
3 107-358 of Stat3 (SEQ ID NO:14), residues 107-342 of Stat3 (SEQ ID
4 NO:15), residues 107-282 of Stat3 (SEQ ID NO:16), residues 107-249 of Stat3
5 (SEQ ID NO:17), residues 130-358 of Stat3 (SEQ ID NO:18), residues 130-
6 342 of Stat3 (SEQ ID NO:19), residues 155-282 of Stat3 (SEQ ID NO:20),
7 residues 155-249 of Stat3 (SEQ ID NO:21), residues 155-377 of Stat3 (SEQ
8 ID NO:22), residues 193-377 of Stat3 (SEQ ID NO:23); residues 249-377 of
9 Stat3 (SEQ ID NO:24); residues 282-377 of Stat3 (SEQ ID NO:25), residues
10 1-154 of Stat1 (SEQ ID NO:11), residues 107-374 of Stat1 (SEQ ID NO:12),
11 and residues 375-750 of Stat1 (SEQ ID NO:13).

1 66. Stat3 mutants Stat3(L148A) (SEQ ID NO:30), Stat3(V151A) (SEQ ID
2 NO:31), or Stat3(T346A, K348A, R350A) (SEQ ID NO:29).

1 67. The Stat3 fragment of claim 65 comprising a GST fusion sequence or an
2 epitope tag.

1 68. A polynucleotide encoding a Stat fragment of claim 65.

1 69. A polynucleotide encoding the Stat3 mutant of claim 66.

1 70. A cell transiently expressing a mutant Stat3 protein of claim 66.

1 71. A Stat-interaction fragment of c-Jun residues 1-104 (SEQ ID NO:26) or
2 residues 105-334 (SEQ ID NO:27).

1 72. A cell transiently expressing a mutant c-Jun fragment of claim 71.

1 73. The method of claim 1 or 26 wherein said agent is capable of modulating
2 cellular transformation.

1 74. The method of claim 73 wherein said agent inhibits cellular transformation.

1 75. The method of claim 73 wherein said agent promotes cellular transformation.

1 76. A method for identifying a mutant Stat protein capable of modulating the
2 transcriptional cooperation between a Stat protein and a transcription factor
3 comprising the steps of:
4 (a) providing a transformed cell line;
5 (b) transfecting said cell line with a Stat mutant suspected of
6 interfering with the interaction between said Stat and a
7 transcription factor;

- (c) examining said cell line for evidence of alteration of transformation in contrast to said cell line transfected with the wild-type Stat;
- (d) identifying a mutant capable of modulating the transcriptional cooperation between a Stat protein and a transcription factor as one which alters the transformation of said cells.

77. The method of claim 76 wherein said evidence of alteration of transformation is change in morphology on soft agar.